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Establishing regional specificity of neuroestrogen action

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ABSTRACT

The specificity of estrogen signaling in brain is defined at one level by the types and distributions of receptor molecules that are activated by estrogens. At another level, as our understanding of the neurobiology of the estrogen synthetic enzyme aromatase has grown, questions have emerged as to how neuroactive estrogens reach specific target receptors in functionally relevant concentrations. Here we explore the spatial specificity of neuroestrogen signaling with a focus on studies of songbirds to provide perspective on some as-yet unresolved questions. Studies conducted in both male and female songbirds have helped to clarify these interesting facets of neuroestrogen physiology.

1. Introduction

The past 40 years have seen rise to an enormous body of research demonstrating that the expression and activity of the estrogen synthetic enzyme aromatase is a conserved property of the vertebrate brain. These studies have demonstrated brain aromatase in an extraordinary diversity of species from virtually every vertebrate lineage, that aromatase can be expressed in a wide variety of neural circuits, that aromatase is expressed in a diversity of cell types, is present in neurons in somata, processes and in terminals, that the enzyme is subject to diverse regulatory mechanisms, and that the substrates for brain aromatization can arise from peripheral or central steroidogenesis. On top of this, we also know estrogen receptors (ER) can be distributed in numerous subcellular locations, from nuclei to membrane sites potentially quite distal to ER-positive somata. The breadth and importance of this rather complex field of neuroestrogen synthesis has recently been covered quite extensively (Balthazart and Ball, 2013; Micevych, 2012), but questions remain. A crucially important set of issues involve how, within the brain's highly heterogeneous steroidal environment, is spatial specificity of estrogen provision achieved (Schmidt et al., 2008). Here, we focus on three outstanding questions and on studies of neuroestrogen synthesis in the songbird brain to provide perspective on some as-yet unresolved questions.

A brief description of the traditional view of brain aromatization is needed to launch this discussion. The bulk of early studies on the role of brain aromatase focused on males and established the principle that testosterone secreted by the testes reached the brain where, in or near discrete regions that expressed aromatase, locally produced 17β-estradiol masculinized (or defeminized) neural circuits developmentally and, in adults, activated circuits to produce a masculine behavioral phenotype. The key features that are crucial here are (a) that the testosterone was produced peripherally in males in which there was little or no peripherally produced neuroactive estradiol (a condition that stood in stark contrast with females that secreted ample estradiol from their ovaries to activate feminine behaviors); (b) steroids were thought to diffuse liberally in brain so gonadal testosterone was available to the whole brain but was only locally converted to estradiol where aromatase was found; (c) that aromatase was close to target neurons expressing receptors for estrogens; and (d) during development, and possibly also in adults, the brains of both males and females were protected from inappropriate exposure to any peripherally produced estrogen, that is ovarian or maternal, by binding proteins in blood. Whereas much of this foundational work remains undisputed, recent studies regarding direct neurosteroidogenesis, diverse functions for brain aromatase outside of the control of reproductive behavior and physiology, and the rapid neuromodulatory roles for estrogens, force expansion of some of these basic concepts. The following are three questions that are unresolved but which bear strongly on our concepts of the spatial specificity of neuroestrogen action:

(1) When aromatase is present in brain *and* estradiol is available from the periphery, how do neural estrogen targets restrict or balance their responses to peripheral vs centrally produced estradiol?
(2) As steroids are lipophyllic molecules, they are often conceived as diffusing relatively freely in brain. How then are estrogen actions spatially restricted near

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aromatase-expressing cells? In other words, how do estrogen-dependent neural circuits preserve the spatio-temporal fidelity of the estrogen signal?

(3) The two previous questions focus solely on neuroactive estrogens. As estrogen synthesis requires androgenic substrates, how do neural circuits balance their access to peripherally derived androgens with those potentially derived from nearby neurosteroidogenic circuits?

1.1. Songbird brain aromatase

As we will highlight work on songbirds, a description of brain aromatization in these birds is essential before expanding on these basics in subsequent sections. Much early work on avian aromatase focused on non-songbirds, like doves and quail (Schlinger and Balthazart, 2013), and showed that, as in some mammals, aromatase was expressed at its highest levels in regions of the hypothalamus and other parts of the animal's "social brain" (Goodson, 2005). Songbirds differ taxonomically from these other species and are attractive animal models in neurobiological research because they (a) sing complex songs, acoustic signals used for reproductive and non-reproductive communication; (b) they learn these songs from their father (or other tutor) developmentally; and (c) they possess a unique complex set of sex-steroid sensitive neural circuits that enable song learning and production. Investigation of brain aromatase in these birds showed that the enzyme was expressed widely, in "social brain" sites as in non-songbirds, as well as in many other brain regions (Saldanha et al., 2013). Aromatase in the songbird brain was present in neuronal somata, as well as in processes that projected locally or to more distal brain regions that lacked somal aromatase (Saldanha et al., 2000). Aromatase-positive synaptic terminals were seen to contact aromatase-positive and negative dendrites, axons and somas (Peterson et al., 2005).

One site of especially high aromatase in songbirds was found in the caudal nidopallium. Recent studies demonstrate that estrogens formed in this region, that includes an auditory processing area called NCM, fluctuate independently of estrogens in blood and do so in response to appropriate auditory and/or visual stimuli (Remage-Healey et al., 2012, 2008). These estrogens produced in the NCM rapidly boost local auditory responses to song stimuli and appear to improve auditory perception (Remage-Healey et al., 2010).

Another site of especially high aromatase in several songbird species is the hippocampus (HP) (Saldanha et al., 1998). This region, strongly associated with spatial learning and memory capabilities (Patel et al., 1997; Watanabe and Bischof, 2004), is structurally elongated rostro-caudally in birds and caudally lies just dorso-medially to the NCM, though separated by the lateral ventricle. Estrogens impact spatial learning and memory in many species, including in songbirds (Oberlander et al., 2004; Rensel et al., 2013), and can do so by direct actions on the HP (Bailey et al., 2013).

Aromatase is constitutively expressed in neurons in the brains of mammals and birds, though in teleost fish, radial glia can be the dominant aromatase-positive cell (Forlano et al., 2001; Pellegrini et al., 2013). After neural injury in mammals and birds, aromatase-expression is up-regulated in reactive astrocytes (in mammals) and in astrocytes and radial glia (in songbirds) (Duncan et al., 2013; Peterson et al., 2004; Spence et al., 2009). Studies of songbirds show that injury-induced aromatase limits the spread of the injury and assists with some neural repair (Wynne and Saldanha, 2004; Saldanha et al., 2005; Peterson et al., 2007).

Thus, in the uninjured songbird brain, aromatase is present in numerous sites with known reproductive functions as well as in sites not traditionally associated with reproduction. Some of these estrogen-dependent regions are in close proximity to one another, like the NCM and the HP. Some brain regions contain aromatase-positive somata, fibers and terminals whereas other regions, or even sub-regions, contain just aromatase-positive fibers and terminals. Superimposed on this distribution, injury induces aromatase in new populations of glial cells. With this background, we now return to address questions about the spatial specificity of estrogen action on brain.

Question 1: When aromatase is present in brain *and* estradiol is available from the periphery, how do neural estrogen targets restrict or balance their responses to peripheral vs centrally produced estradiol?

The ovaries of female vertebrates secrete estradiol, in regular as well as periodic episodes, from sexual maturity until they become post-reproductive, and these estrogens are seen as activating neural circuits that yield the feminine behavioral phenotype. However, in the face of a peripheral supply of estrogens, there is still evidence that brain aromatase in females is functional, suggesting that central-produced and peripherally-produced estrogens have unique functions in brain. At least two examples have been demonstrated in songbirds. In one case, estrogen-dependent, auditory-induced neuronal responses in the NCM of female zebra finches are inhibited by local application of fadrozole, an inhibitor of the aromatase enzyme (Remage-Healey et al., 2012). In a second case, estrogen-dependent gene regulation associated with neural repair/neuroprotection in females is reduced by neural application of an aromatase inhibitor (Walters and Saldanha, 2008). An important common theme is that functional estrogen synthesis was inhibited locally, within the brain, in these two observations in females. Consequently, in both of these cases, despite the likelihood that estrogens are present in blood, ovarian estrogens seem to play little role in these neural estrogen-dependent actions.

Results like these raise several questions. Are ovarian estrogens somehow restricted from reaching some, but not all, neural target sites? Are the concentrations of estrogen in blood sufficient to activate some neural circuits, like those involved in female sexual behavior, but not others, such as circuits involved in auditory processing or in neural repair? Are these examples of widespread and ever present physiological systems that routinely function to allow circulating estrogens to activate only some neural pathways while allowing locally produced estrogens to only activate other cells and circuits?

Circulating steroids are thought to readily cross the blood–brain-barrier (Pardridge, 1981; Banks, 2012) or are thought to circulate bound to proteins that restrict or enhance their delivery to the brain. According to the free hormone hypothesis (Mendel, 1989), only 'free' or unbound steroid hormone can reach receptors on target tissues. Recent work with sex hormone binding globulins (SHBGs, which bind androgens and estrogens) and corticosteroid binding globulins (CBGs, which bind corticosteroids, progesterone, and testosterone) suggests that, in contrast to this view, hormone binding globulins act to maintain access of steroid to receptors through multiple mechanisms. First, CBGs in mammals reduce clearance rates of circulating corticosterone, ensuring that stress-induced concentrations are adequate to reach targets in the brain and impact behavior (Minni et al., 2012; Moisan et al., 2013). Second, both CBGs and SHBGs are locally produced in several mammalian brain regions (Herbert et al., 2003, 2006; Jirikowski et al., 2007; Mopert et al., 2006). SHBGs localized in the brain appear to facilitate uptake or sequestration of hormones, possibly through interaction with the ERβ receptor (Caldwell et al., 2007). Finally, uptake of estradiol in the hamster ovary is dependent on the presence of SHBG (Caldwell and Jirikowski, 2013). Therefore it appears